510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

k112120

B. Purpose for Submission:

Clearance of a D-dimer assay, controls and calibrators

C. Measurand:

D-dimer

D. Type of Test:

Latex enhanced immunoturbidimetric assay

E. Applicant:

Diazyme Laboratories

F. Proprietary and Established Names:

Diazyme D-Dimer Assay Kit
Diazyme D-Dimer Assay Calibrator Set
Diazyme D-Dimer Assay Control Set

G. Regulatory Information:

1. Regulation section:

   21 CFR §864.7320, Fibrinogen/fibrin degradation products assay

2. Classification:

   Class II

3. Product code:

   GHH, Fibrin Split Products
   JIT, Calibrator, Secondary
   DAP, Fibrinogen and Fibrin Split Products, Antigen, Antiserum, Control

4. Panel:

   81 Hematology

H. Intended Use:

1. Intended use(s):

   The D-Dimer Assay is for the quantitative determination of fibrinogen/fibrin degradation products (D-Dimer) in human plasma. Measurement of D-Dimer is used as an aid in detecting the presence of intravascular coagulation and fibrinolysis. For *in vitro* diagnostic use only.

   The D-Dimer assay calibrator set is intended for use in the calibration of the D-Dimer assay only. For *in vitro* diagnostic use only.

   The D-Dimer Assay Control Set is intended for use as quality controls for the Diazyme D-Dimer assay only. For *in vitro* diagnostic use only.

2. Indication(s) for use:

   Same as Intended use

3. Special conditions for use statement(s):

   Not applicable

4. Special instrument requirements:

   Roche Modular P

I. Device Description:

The Diazyme D-Dimer Assay is a quantitative assay for the determination of fibrin degradation products in human citrated plasma.

Each assay kit consists of Reagent 1 (Ready-to-use Tris-buffer) and Reagent 2 (Ready-to-use suspension of anti-human D-dimer mouse monoclonal antibody coated latex particles). The system consists of the assay, calibrators and 2 controls, however the calibrator and controls are provided separately.

The Control set consists of one high and one low level lyophilized control in 1.0 mL vials.

The Calibrator set consists of 5 levels of lyophilized calibrators in 1.0 mL vials. An additional blank calibrator (saline) is required but not provided.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Roche TINA-QUANT D-Dimer System

2. Predicate 510(k) number(s):

   k062203

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended Use	For the quantitative determination of fibrinogen/fibrin degradation products in human plasma.	Same
Principle	Immunoturbidimetric	Same
Type of test	Quantitative	Same
Reagents	Buffer and anti-D-dimer antibody coated latex beads.	Same
Controls	Bi-level controls, lyophilized (Control concentrations supplied in the certificate of analysis)	Same

Differences		
Item	Device	Predicate
Indication for use	As an aid in detecting the presence of intravascular coagulation and fibrinolysis. Not for exclusion of deep vein thrombosis (DVT) and pulmonary embolism (PE)	In conjunction with non-high clinical probability assessment, a normal (<0.5ug FEU/mL) result excludes deep vein thrombosis (DVT) and pulmonary embolism (PE) with high sensitivity.
Analyte measured	D-Dimer	D-dimer and x-oligomers
Sample Matrix	Citrated plasma	Citrated plasma and Li heparin
Instrument Platform	Roche Modular P	Roche/Hitachi MODULAR analyzers and cobas c analyzers.
Calibrator	5 different levels of calibrator supplied ready for use upon resuspension. Calibrator concentrations supplied in the certificate of analysis. The sixth level of calibrator is a blank.	One calibrator supplied for creation of a calibration curve by dilution to a calibration curve consisting of five calibrator levels and one blank.

K. Standard/Guidance Document Referenced (if applicable):

CLSI EP5-A2, Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline- Second Edition

CLSI EP6-A, Evaluation of the Linearity of Quantitative Measurement Procedure; Approved Guideline

CLSI EP7-A, Interference Testing in Clinical Chemistry; Approved Guideline

CLSI EP9-A2, Method comparison and Bias Estimation Using Patient Samples; Approved Guideline- Second Edition

CLSI EP17-A, Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline

CLSI C28-A3, Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline- Third Edition

L. Test Principle:

This assay measures the fibrin degradation product D-dimer, which is present in the blood after a blood clot is degraded by fibrinolysis. The Diazyme D-Dimer Assay is a latex enhanced immunoturbidimetric assay in which anti-D-dimer coated latex particles bind to the D-dimer proteins present in the sample, causing agglutination. The degree of turbidity is assessed optically and is proportional to the amount of D-dimer present in the sample. The actual amount of D-dimer is determined from a calibration curve.

M. Performance Characteristics (if/when applicable):

1. <u>Analytical performance:</u>

   All analytical studies were performed on the Roche Modular P instrument.

   a. Precision/Reproducibility:

   Precision was evaluated according to CLSI EP5-A2. Three levels of D-Dimer controls (0.97, 2.99 and 7.47 µg/mL FEU), one unaltered pooled plasma sample (0.6 µg/mL FEU) and two plasma pools spiked with D-Dimer stock solution at levels of 2.41 µg/mL and 7.47 µg/mL FEU were tested. Samples were tested in duplicate, with 2 runs per day over 20 working days using three reagent and three calibrator lots. Acceptable criteria of CV≤ 8% for analyte concentrations greater than 0.05 µg/mL FEU were met. The table below represents results for the combined data.

Plasma Samples Within-Run Precision

Samples	Level 1 0.60 µg/mL FEU	Level 2 2.41 µg/mL FEU	Level 3 5.88 µg/mL FEU
Data Points N	240	240	240
Mean (µg/mL FEU)	0.60	2.41	5.88
SD (µg/mL FEU)	0.03	0.05	0.08
CV%	5.0%	2.0%	1.4 %

Plasma Samples Total Precision

Samples	Level 1 0.60 µg/mL FEU	Level 2 2.41 µg/mL FEU	Level 3 5.88 µg/mL FEU
Data Points N	240	240	240
Mean (µg/mL FEU)	0.60	2.41	5.88
SD (µg/mL FEU)	0.04	0.07	0.19
CV%	4.9%	2.8%	3.0%

Control Samples Within-Run Precision

Samples	Level 1 0.97 µg/mL FEU	Level 2 2.99 µg/mL FEU	Level 3 7.47 µg/mL FEU
Data Points N	240	240	240
Mean (µg/mL FEU)	0.97	2.99	7.47
SD (µg/mL FEU)	0.03	0.05	0.11
CV%	2.9%	1.6%	1.4%

Control Samples Total Precision

Samples	Level 1 0.97 µg/mL FEU	Level 2 2.99 µg/mL FEU	Level 3 7.47 µg/mL FEU
Data Points N	240	240	240
Mean (µg/mL FEU)	0.97	2.99	7.47
SD (µg/mL FEU)	0.04	0.08	0.27
CV%	4.4%	2.8%	3.6%

A reproducibility study was performed at three external testing sites. Testing included three reagent and calibrator lots. Four samples of citrated patient plasmas ranging from 0.36 µg/mL to 7.2 µg/mL FEU were tested in duplicate for 2 runs per day for 5 non-consecutive days. Statistical evaluation was applied to identify sources of variability. Within-run, between-run, between-day, between-lot (operator, site and instrument) and total precision were calculated. Results met acceptance criteria for CV <10%; <0.5 µg/mL FEU SD 0.05 µg/mL FEU, as summarized in the table below.

Panel Members	Mean µg/mL FEU	Within-Run		Between-Run		Between-day		Between-lot		Total	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Patient 1	0.36	0.03	8.9%	0.03	8.3%	0.03	7.6%	0.03	7.1%	0.04	11.5%
Patient 2	1.06	0.04	3.8%	0.04	3.6%	0.08	7.4%	0.09	8.3%	0.09	8.3%
Patient 3	3.53	0.10	2.9%	0.14	3.9%	0.11	3.1%	0.02	0.7%	0.17	4.7%
Patient 4	7.20	0.12	1.6%	0.23	3.1%	0.17	2.4%	0.11	1.5%	0.25	3.5%

b. Linearity/assay reportable range:

Linearity was demonstrated following CLSI EP6-A. An 11-point linearity set was prepared from a plasma sample containing 8.3 µg/mL FEU by dilution of the plasma sample with saline at varying ratios. Samples were tested in triplicate, and demonstrated linearity for D-Dimer concentrations from 0.003 to 8.6 µg/mL FEU. The acceptance criteria allowable total error of <10% was met.

A hook effect study was performed by testing dilutions of a high concentration D-dimer stock solution to levels ranging from 0.48 µg/mL to 30 µg/mL FEU. No hook effect was observed in the claimed linear range.

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

Value assignment for calibrators and controls:
Diazyme D-Dimer calibrators are derived from human serum and value assignment is based on the predicate assay Roche TINA QUANT D-Dimer system.
Diazyme D-Dimer controls are traceable to in-house standards. The Diazyme D-Dimer reagents and calibrators are used to determine the mean value of the newly prepared controls. The expected range is calculated as ± 15% of the mean value as determined by testing with one Roche Modular P instrument, two lots of reagents and three vials from each level over two testing days.

Reagent stability
Reagent shelf stability
All stability testing was performed using the Roche Modular P instrument. The reagent real time stability claim at 2-8°C is based on ongoing real time stability data available for 3 lots of reagent, 9 months at time of clearance. The sponsor also provided data from accelerated stability studies on 2 lots of reagents tested at 37°C and 25°C. At specified times, assay kits were evaluated by testing D-Dimer controls ranging from 1.1 µg/mL to 4.5 µg/mL FEU after calibration. Results demonstrated that the reagents were stable for 14 days at 37°C and for 2 months at 25°C.

Acceptance Criteria of <15% control recovery change from Day 0 value were met.

On-board reagent stability
On board reagent stability was performed by testing 3 levels of controls with one lot of reagents. Results demonstrated on-board stability of at least 6 weeks (Acceptance criteria ≤10% control recovery change from Day 0 value were met).

Calibrator stability
Calibrator shelf stability
Real time stability data for storage of three calibrator lots at 2-8 °C available at time of clearance supported calibrator shelf life of 9 months. In addition, sponsor provided data from accelerated studies that tested two calibrator lots stored at 37°C or 25°C. Results demonstrated that the lyophilized calibrators were stable for 21 days at 37°C and for 3 months at 25°C by meeting the acceptance criteria of ≤ 10% calibrator recovery from Day 0
Reconstituted calibrator stability
Reconstituted calibrator stability demonstrated at least 21 days stability at 2-8ºC. Acceptance criteria of ≤ 10% calibrator recovery from Day 0 were met in each of these studies.

Calibration curve stability
Three levels of controls from one lot were tested repeatedly after calibration at day 0 over a period of 28 days. This calibration stability study demonstrated a curve stability of 4 weeks (Acceptance criteria ≤10% control recovery change from Day 0 value were met).

Control stability
Control shelf stability
Real time stability data for storage of three control lots at 2-8 °C available at time of clearance supported calibrator shelf life of 9 months. Accelerated study data on two control lots stored at 37°C and 25°C and tested in duplicate at specified times demonstrated that two lots of controls are stable for 19 days at 37ºC, and 3 months at 25ºC. Acceptance criteria of ≤ 15% calibrator recovery from day 0 were met in each of these studies.

Stability of reconstituted controls
Reconstituted control stability was determined by repeat testing of 2 control lots for up to 28 days for storage at 2-8ºC. Acceptance criteria of ≤ 15% calibrator recovery from day 0 were met in each of these studies. Likewise, these criteria were met for open-vial stability of up to 14 hours at room temperature.

Sample stability
Two citrated plasma samples were tested daily after storage at 2-8ºC. The

acceptance criteria of <10% change from day 0 were met for storage up to 4 days. One freeze- thaw cycle between day 3 and 4 further indicated freeze-thaw stability. Testing after 3 months of storage at -20ºC demonstrated samples were stable and met the same acceptance criteria.

Sample freeze thaw stability
Freeze thaw stability was further demonstrated by testing 10 plasma samples ranging from 0.32 to 5.01 µg/mL FEU that had been frozen at -20ºC for three days. Results were compared to initial assessments of the fresh samples prior to freezing. Samples are within the predetermine acceptance criteria of 10% or ± 0.1 µg/mL FEU for samples below 0.5 µg/mL FEU.

d. Detection limit:

LOB, LOD, and LOQ of the Diazyme D-Dimer Assay were determined according to CLSI EP17- A: Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline, using 7.5% Bovine Serum Albumin (BSA) in saline as the true blank, and five citrated plasma samples on the Roche Modular P. For LOB determination, the citrated samples were diluted 1/100, and 12 replicates of each were assayed. Five blank samples were tested in quadruplicate on each of three testing days for a total of 60 observations and the LOB was determined to be 0.06 µg/mL FEU. Similarily, 5 citrated plasma samples were diluted 100 fold with saline containing 7.5% BSA. Each sample was tested in 12 replicates. The LOD was determined to be 0.09 µg/mL FEU. Analysis of repeated testing of five diluted citrate plasma samples ranging in concentration from 0.02 µg/mL to 0.93 µg/mL FEU with 5 runs in 8 replicates per run by EP evaluator-8 fitted modeling calculated an LOQ of 0.15 µg/mL FEU.

e. Analytical specificity:

Testing was performed by evaluating the effects of increasing concentrations of potential interfering substances on assay performance by testing spiked two plasma samples of high and low D-dimer concentration spiked with increasing concentrations of interferent in triplicate. The interfering substances of triglyceride, ascorbic acid, bilirubin, conjugated bilirubin, hemoglobin, rheumatoid factor, heparin, and human anti-mouse antibody (HAMA) showed no significant interference (< ± 10%) up to the concentrations summarized below.

Interferent	Concentration
Triglyceride	1000 mg/dL
Ascorbic Acid	176 mg/dL
Bilirubin	40 mg/dL
Bilirubin Conjugated	40 mg/dL
Hemoglobin	1000 mg/dL
Rheumatoid Factor	100 IU/mL
Heparin	1.5 IU/mL
HAMA	490 ng/mL

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. Method comparison with predicate device:

The performance of the Diazyme D-Dimer assay on the Roche Modular P instrument was compared to the predicate device (Roche TinaQuant D-Dimer Assay (k062203)). Testing was performed on citrated plasma samples from the intended use population (patients in intensive care, trauma patients and post-operative patients). While one of the sites have unique sample sets, sites 1 and 3 share most samples. A total of > 40 patient samples were tested with both assays at each site. A total of 128 measurements were performed with each instrument with 88 unique samples. Bootstrap analysis was performed to negate the effects of repeat testing of samples. Results met the acceptance criteria of slope = 1.0 ± 0.1; $r^2 \geq 0.90$ and intercept ± 0.15.

b. Matrix comparison:

Not applicable

3. Clinical studies:

a. Clinical Sensitivity:

Not applicable

b. Clinical specificity:

Not applicable

c. Other clinical supportive data (when a. and b. are not applicable):

Not applicable

4. Clinical cut-off**:**

Not applicable

5. Expected values/Reference range:

120 apparently healthy adults were tested in duplicate using the Diazyme D-Dimer assay according to CLSI C28-A3. Testing was performed on the Roche Modular P instrument. Results demonstrated the expected normal range of < 0.5 µg/mL FEU in 90% of the tested population.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.